

CUTANEOUS MINERAL LOSS
IN PREADOLESCENT GIRLS

by

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INTRODUCTION

It has been recognized since the late 1940's that sweat contains most of the nutrients found in blood plasma. Among the nutrients found in sweat are the essential minerals, calcium, copper, iron, magnesium, manganese, sodium, potassium and zinc. Although these minerals are recognized as components of sweat, the amounts lost cutaneously are still uncertain.

Some research has been done with adult subjects to quantitatively determine mineral loss of Ca, Na, and Fe but these data are confusing and inconsistent. Variations in techniques of collecting and analyzing sweat samples have resulted in a wide range of reported values. Data on the skin loss of the trace minerals, Zn, Mn, and Cu are scarce.

The work that has been done with cutaneous mineral losses has been with adults and little research has been done with other age groups. Data are still missing as to the amounts of the essential minerals retained by the growing child. Because recommended daily allowances are based on balance study data, it is important for balance study data to include all possible routes of nutrient excretions. Results of experiments by Johnston et al. (17) indicate that the intake of calcium and iron should be increased to cover the skin losses of the average adult woman engaged in normal activity under conditions of average

temperature and humidity. Other researchers found that cutaneous mineral excretions can affect mineral balances. Consolazio et al. (8) found that positive potassium balances were changed to negative values and iron balances were cut in half when sweat losses were included in calculating balances. Issaksson et al. (16) observed that positive Ca balances were changed to zero or negative values when sweat losses were considered.

Because of the paucity of data available on cutaneous mineral losses of children, this research project was designed to determine the amounts of Ca, Cu, Fe, Mg, Mn, K, Na, and Zn lost in forearm sweat thus providing information about cutaneous mineral losses in children. This research was planned to provide information to assist in the determination of mineral requirements.

REVIEW OF LITERATURE

Nutrient Loss through the Skin

Water is continually vaporized from the skin surface to maintain the homothermic condition of the body. Water lost in this manner is known as "insensible loss of water". When this continual vaporization is insufficient to rid the body of excess heat, then the sweat glands become activated to pump water to the skin surface to further increase heat loss. As water is lost through the skin, by either method, it carries with it certain nutrients. For this reason cutaneous nutrient excretions should not be ignored in balance studies or the nutrient requirements and allowances estimated from balance study data may be too low for optimal health.

The amount of water and nutrients lost through the skin depends on the rate of perspiration which is influenced by: (1) environmental temperature; (2) work or activity; and (3) stress or tension. The sweat glands which regulate the rate of perspiration can be activated by heat, chemicals or nervous stimulation. Under conditions of high temperature and humidity where high sweat rates were required, Collins and Weiner (3) found that the sweat glands reached a limit of performance and then the sweat rate began to decrease. The rate of sweating also varies with the individual and his adaption to the environment and the area of the body (3, 27).

Comar and Bronner (5) reported that the solid content of sweat ranged from 0.3-1.0% and about 50% of the solid content was inorganic. Although it is known that minerals are lost through the skin, information is still lacking as to what extent dermal nutrient losses affect mineral requirements and balance study data.

Methods of Sweat Collection

One reason that reliable information is not available on cutaneous mineral losses is that the area and method of collection varies among researchers. Collection techniques that have been used to obtain sweat samples include: (1) collecting sweat from various parts of the body by rubbing the edge of a beaker across the skin; (2) collecting sweat from the forearm in an impermeable glove or bag; and (3) collecting sweat from the whole body. The data collected by different methods vary. Because collections from the forearm are most convenient and because an impermeable bag helps prevent contamination, the arm-bag method is most frequently used to collect sweat samples. In the arm-bag method the minerals excreted from the forearm are used to extrapolate total body mineral loss. There is controversy, however, whether or not arm-bag sweat is a reliable indicator of whole body sweat. Consolazio et al. (9) and Van Heyningen (25) reported that sweat collected from the forearm in an impermeable bag is more concentrated than

sweat collected from other parts of the body. In the study by Consolazio et al. (9) the calcium excreted in the arm sweat was a reliable indicator of the calcium content of whole body sweat only if the total weight of sweat lost through the skin was greater than 16 g. If sweat loss was less than 16 g, the calcium content of whole body sweat was only 71-76% of the calcium content of arm sweat. Van Heyningen and Weiner (25) observed that chloride, urea, and lactate were higher in arm sweat than in whole body sweat. Issaksson et al. (16) found arm sweat to be a reliable indicator of total body sweat. There are other data available in support of both sides (6, 11, 18).

Cutaneous Excretion of Iron

Because of the limited gastrointestinal absorption of iron, all possible means of excretion should be included in iron balance studies. Most of the iron lost through the skin is exfoliated cells (1), but small amounts are found in filtered cell-free sweat. Collins et al. (4) found that young adult men lost amounts of iron equivalent to 0.5% of their daily iron intake through the urine and equivalent to 1.0% of their daily intake through cell-free sweat. Although the amounts lost are small, the data indicate that twice as much iron was lost through the skin as was lost through the urine. Further experiments on young adult men by Wheeler et al. (28) indicated that 3% of the absorbed

iron is excreted in the cell-free sweat and the amount is always greater than the urinary iron output. Collins et al. (4) found that his subjects lost approximately 0.2 g of iron per day through the skin in a comfortable environment and about double this in a hot environment. Mitchell and Hamilton (20) also used young adult men to study iron loss through the skin and found a loss of 0.27 mg/hr in a comfortable environment and approximately five times this amount, or 1.29 mg/hr, in a hot environment. In their study, sweat samples were collected by rubbing the rim of a beaker across the chest of the subjects which may have caused excessive exfoliation of dermal cells. Consolazio et al. (8) found the daily iron balances of 3 healthy young men to be approximately cut in half when the sweat losses were considered in calculating the balance. Dermal iron loss data for age groups other than adult men are scarce.

The data on iron intake and iron excretion through the skin is varied. Johnston et al. (17) found no evidence that increased intake of iron increased the amount excreted in the sweat. Hussain and Patwardham (15) found an increase in losses through the skin when iron therapy was given. Mitchell and Hamilton (20) also studied the effect of iron intake on the concentration of iron in sweat but did not establish any clear cut effect.

Cutaneous Excretion of Magnesium

Some work has been done to determine the skin loss of magnesium in preadolescent girls. Ryan (22), using pre-adolescent girls consuming a constant intake of Mg, found a loss of about 20.4 mg/day through the skin. This was obtained using the whole body technique. Consolazio et al. (8) found the concentration of Mg in sweat to range from 0.61-0.64 mg/100 ml of sweat lost in young adult men and that the Mg balances were not greatly affected by including sweat losses in the balance data. Results obtained by Mitchell and Hamilton (20) using young adult men found Mg in sweat to vary from 0.04 to 0.40 mg/100 ml of sweat.

Cutaneous Excretion of Calcium

Concentrations of calcium in sweat have been found to be great enough to be considered in determining Ca balances (7, 9, 17). Issaksson et al. (16) found that including the sweat losses of Ca affected Ca balances by changing positive values to zero or negative values. Because of the findings and conclusions of these and other researchers, the National Research Council now includes cutaneous Ca losses in establishing recommended daily allowances for adults (11). The data available on cutaneous loss of Ca in children are scarce and the allowances for the preadolescent group must, therefore, be extrapolated from adult values. Ryan (22), working in this laboratory, found an average

loss of 72.7 mg of calcium per day through the skin of pre-adolescent girls. Some investigators (7, 17) feel that the Ca loss through the skin could affect Ca requirements in areas where the climate is hot and intakes should be increased for people living in these areas. Walker and Richardson (26) investigated this possibility by studying the growth rate of Zulu children from both a hot and a temperate climate. They found no difference in height and weight which they believed indicated that Ca intakes did not need to be increased to cover greater skin losses in hot climates.

Cutaneous Excretion of Copper and Manganese

Few data are available on the copper and manganese content of human sweat. Mitchell and Hamilton (20) reported that adult men lost approximately 5.8 mcg of Cu and 6.0 mcg of Mn per 100 ml of sweat. Their collections were made by rubbing the edge of a beaker over the skin of the subject while the subject was in an atmosphere of controlled temperature and humidity.

Cutaneous Excretion of Potassium

Although the data on cutaneous potassium losses are limited, it seems that skin losses could affect K balance data. Consolazio et al. (8) found that the K balances of three young men were positive when sweat losses were not

included but negative when sweat losses were included. Collins et al. (4) also studied the cutaneous K loss of young men and found that about 10% of the normal K intake was lost through the skin. In the analysis of crude sweat samples acquired by the thermal stimulation of subjects, Seutter et al. (24) found K values to range from 5-59 m Eq/L.

Cutaneous Excretion of Sodium

It long has been realized that excessive sodium losses because of profuse sweating can affect the homeostatic condition of the body. Because Na has a higher concentration in sweat than other minerals it has been researched more thoroughly. Both increased environmental temperature and increased activity have been shown to increase Na loss through the skin. Collins et al. (4) found that six young men lost an average of 24 m Eq of Na per day in a comfortable environment and an average of 51 m Eq per day in a hot environment. Wheeler et al. (28) found that the sweat loss of Na of six adult men was equivalent to 6% of the intake during normal activity and twice this amount when activity was increased. The effect of sweat rate on Na excretion can be seen by comparing the experiments of Consolazio and Freyberg. Consolazio et al. (8) found losses of 0.601 g of Na/hr in men exposed to temperatures of 38 degrees centigrade. Under conditions where sweating

was prevented, only 71 to 209 mg of Na were lost in two men during a twenty-four hour period (12). From these data it is evident that high temperature and humidity which induce profuse sweating can result in significant Na losses.

Controversy still exists about the amounts of minerals lost through the skin and their effect on mineral balances. It is only through additional research that this controversy will be solved. Information on age groups other than adults is badly needed. Research dealing specifically with the cutaneous mineral loss of children is necessary to determine mineral retention in the growing child.

EXPERIMENTAL PROCEDURE

Subjects

The subjects were fifteen girls participating in a metabolic study conducted by the Department of Human Nutrition and Foods under a grant from the National Institute of Health. The ages of the subjects ranged between 7 years and 11 months and 9 years and 5 months with a mean age of 8 years and 7 months. Age, height, and weight for each subject at the beginning of the study are given in Table 1. Prior to the study all subjects were required to pass a physical examination to insure normal health and development.

Subjects were housed in university dormitory facilities converted to a metabolic unit and were fed in a departmental diet kitchen. The subjects were given precisely weighed quantities of food and encouraged to consume all food. If for any reason all the food was not consumed, the uneaten portion was weighed and recorded. Supervision was provided at all times to insure compliance to the metabolic study.

Procedure of the Metabolic Study

The metabolic study began July 12, 1973, and ended August 15, 1973. This 35-day study was divided into four periods (Table 2). The first five days were an adjustment period and all subjects consumed approximately 30 g of

Table 1.

Age, height, and weight of the subjects
at the beginning of the study

Subject	Age		Weight (kg)	Height (cm)
	Year	Month		
100	8	8	31.4	132.0
101	9	5	39.5	143.5
102	8	7	25.8	133.5
103	7	11	25.6	128.5
104	8	1	24.1	128.0
105	8	3	31.0	133.0
106	9	3	26.6	139.0
107	8	7	23.0	125.5
108	8	5	23.4	123.5
109	8	8	32.1	137.5
110	8	10	32.8	133.0
111	8	10	31.1	139.0
112	8	4	24.2	127.0
113	8	11	38.2	137.0
114	7	11	24.4	126.5
Mean	8	8	28.9	132.4

Table 2.

Cycle of rotation of subjects
through experimental treatments

Periods	Dates	Dietary Treatment	Subjects
I	July 12-14	A	All Subjects
II	July 15-24	A B C	Group A Group B Group C
III	July 25- Aug. 3	A B C	Group C Group A Group B
IV	Aug. 4-15	A B C	Group B Group C Group A

protein daily. The following thirty days were divided into three-ten day diet periods, the experimental periods.

The diet consisted of foods generally acceptable to the preadolescent age group. Nutrient content of the foods in the diet was determined from the Home and Garden Bulletin No. 72, U. S. Department of Agriculture, revised edition, 1971. The diet consisted of natural foods and vitamin and mineral supplements so that the nutrient intake, with the exception of protein, met or exceeded the National Research Council's Recommended Dietary Allowances. Three different dietary treatments were given which varied in protein content (30, 60, or 90 g/day). Protein intake was varied because nitrogen balance was studied by other researchers during this experiment. Each protein level contained three different menus labeled X, Y, and Z and these menus were fed consecutively during each treatment in the following manner: X, Y, Z, X, Y, Z, etc. The fifteen subjects were randomly divided into three groups (A, B, C) of five subjects each. Each group of subjects was randomly rotated through each treatment in such a manner that all three groups consumed each dietary treatment during the experimental periods.

Temperature and humidity were not controlled. It was felt that data on nutrient excretions collected during normal atmospheric conditions would have more practical application than those collected under artificial

conditions. Temperature and humidity were recorded and are summarized for days that arm sweat was collected (Table 3).

Collection Procedures

Daily food samples were taken for each menu and were composited for each period to be assayed for mineral and nitrogen content. Urine and feces were collected during the last five days of each experimental period for nitrogen determinations. Sweat collections for nitrogen determinations were made from the total body during the last two days of each diet period. Appendix A gives a brief description of the procedure used for whole body sweat collection and analysis. A detailed report of this part of the study will be reported separately (Paula Howat, Doctoral Dissertation, VPISU). Sweat collections for mineral analysis were made immediately following total body collections.

Collections from the whole body were not feasible because of possible contamination from the environment, therefore, the impermeable bag was the method used to collect samples for mineral assay. An impermeable bag protected against environmental contamination and thus should give good results on mineral excretion through the skin. Each subject's arm was wiped off with a damp cloth which had been previously washed in acid and rinsed in ion free water to reduce mineral content. The arm was then

Table 3.

Temperature and relative humidity
on days of arm sweat collection

Period	Date	Temperature °F	Relative Humidity
II	July 25	70.5	86.5%
III	August 4	71.0	74.2%
IV	August 15	67.5	81.0%

rinsed with 100 ml of a 0.005% polyethylene laural alcohol detergent and 200 ml of ion-free water. The forearm was then dried with a low mineral towel. A one-half gallon polyethylene freezer bag was placed on the arm and secured with a rubber band. Prior to being worn by the subject, the freezer bag was specially washed to reduce mineral content. The subject wore the bag for one hour. The bag was then removed and the subject's arm was washed with 100 ml of a 0.005% laural alcohol detergent and a wash cloth. The arm was then rinsed with 200 ml of ion-free water and both the wash and rinse water was saved for analysis. The freezer bag, wash cloth and 100 ml of 0.05% nitric acid were added to the wash solution; the mixture was allowed to equilibrate during a five hour period. After equilibrium had been reached, a 250 ml sample was taken for mineral analysis. Samples for mineral determinations were filtered to remove organic contaminants. In addition to the forearm sweat collection from the subjects, five blank collection bags were also used. Blanks were prepared exactly the way as the samples except the plastic bags used in the blanks were not worn by subjects. The blanks were prepared by soaking a low mineral wash cloth and a plastic bag in a solution of 100 ml of 0.005% laural alcohol detergent, 200 ml of ion-free water and 100 ml of 0.05% nitric acid. The blanks were also allowed to sit for at least five hours to reach

equilibrium and were analysed exactly as the sweat samples.

Treatment of Materials

All towels and wash cloths used for arm sweat collections were treated to reduce mineral content. First they were washed in a dilute soap solution and then rinsed with tap water and dried. They were then soaked overnight in ion-free water and then soaked overnight in a 0.05% nitric acid solution. Following the nitric acid soak they were soaked three times in ion-free water for a period of approximately 16 hours for each soaking. Between each soaking procedure excess water was extracted by wringing. Because of possible contamination from electric dryers or the atmosphere, the towels and wash cloths were not dried after soaking but were kept in plastic bags until used.

The polyethylene freezer bags used for arm sweat collections were treated in the following manner: (1) rinsed with ion-free water and allowed to drip dry; (2) acid rinsed in a 50% nitric acid solution; and (3) rinsed four times with ion-free water.

Nutrient Determinations

The 250 ml sample taken for mineral analysis was filtered and then evaporated to dryness by the application of dry heat. The precipitate remaining after evaporation was wet ashed by the addition of concentrated nitric acid

until all organic material was oxidized. After the organic material was oxidized, 3 ml of concentrated nitric acid and 2 ml of concentrated perchloric acid were added to insure complete oxidation and were evaporated until a white precipitate was produced. The resulting precipitate was then dissolved in 2 ml of concentrated hydrochloric acid and hot water and brought up to a volume of 25 ml. The samples were then assayed on a Perkin Elmer Model 305 (Three Slot Burner Head) Atomic Absorption Spectrophotometer. Food composites for each period were also analyzed for mineral content. The procedure for this analysis will be presented separately (Andhal Raghaven, Masters Thesis, VPISU).

Statistical Analysis of Data

The results were analyzed using an analysis of variance with two variables of classification and repeated measurements. The statistical significance of dietary intakes and different experimental groups were investigated by using a 5% significance level.

RESULTS AND DISCUSSION

Mineral Intake

The mean intake in mg/day of calcium, copper, iron, magnesium, manganese, sodium, potassium and zinc for the three dietary treatments are given in Table 4. These values represent the mean of all menus fed during each treatment for three periods. The intake values were found to determine the effect of intake on mineral excretion in the sweat. Cu, Fe, and Mn did not differ greatly between dietary treatments.

Effect of Intake on Excretion

The average cutaneous mineral excretion from the forearm of three groups of subjects consuming various intakes of each mineral are given in Tables 5 - 7. Table 5 gives the average loss of Ca, Cu, and Mg in forearm sweat. Calcium excretion in forearm sweat from individual subjects ranged from 3.39-35.32 mcg/hr. These two values were found in two different subjects on dietary treatment A. The range of copper excretion for individual subjects was 0.04-0.66 mcg/hr. One subject lost no detectable Mg in the forearm sweat. The highest Mg excreted for any subject was 7.98 mcg/hr. The excretion of 0.00 and 7.98 mcg/hr of Mg was excreted by the same subject (subject 103) on treatments C and A, respectively. This clearly shows the wide variation of

Table 4.
Mineral intake^a during
three dietary treatments

Mineral	Dietary Treatment		
	A	B	C
Calcium	1469.4	1315.6	1142.9
Copper	1.1	1.0	1.2
Iron	30.2	32.0	32.0
Magnesium	172.3	276.8	286.6
Manganese	3.0	2.5	2.5
Potassium	2312.4	2969.2	2672.7
Sodium	2182.7	3092.1	3294.2
Zinc	4.2	8.2	9.7

^aIntake for each mineral is in mg/day

Table 5.

Average excretion^a of calcium, copper, and magnesium
in forearm sweat during three levels of intakes^b
and for three groups of subjects

Subjects	Intake of Ca ^b			Intake of Cu ^b			Intake of Mg ^b		
	1469.4	1315.6	1142.9	1.1	1.0	1.2	172.3	276.8	286.6
Group A	20.595 +10.53 —	10.495 +1.80 —	9.276 +7.64 —	0.126 +0.03 —	0.129 +0.01 —	0.232 +0.11 —	3.080 +2.80 —	0.856 +0.47 —	1.705 +1.30 —
Group B	5.651 +1.62 —	11.544 +7.61 —	10.031 +5.50 —	0.097 +0.03 —	0.109 +0.03 —	0.135 +0.04 —	0.802 +0.26 —	1.590 +0.74 —	0.772 +0.35 —
Group C	7.753 +2.62 —	4.521 +0.80 —	18.335 +2.80 —	0.112 +0.02 —	0.284 +0.23 —	0.144 +0.06 —	0.771 +0.30 —	0.631 +0.30 —	2.283 +0.81 —
Overall Mean	11.333 +8.10 —	8.853 +3.83 —	12.583 +5.00 —	0.112 +0.01 —	0.174 +0.10 —	0.170 +0.05 —	1.551 +1.32 —	1.029 +0.50 —	1.586 +0.76 —

^aMineral excretion is given in micrograms lost per hour + SD

^bIntake is given in milligrams per day

excretion among individuals. The only possible explanation for the high excretion is that this collection was made at the first collection period, and nervous stimulation could have caused an increased sweat rate. In general, the excretions from all subjects were higher during this first collection period. The reason no Mg was excreted by subject 103 on treatment C cannot be explained at this time.

The average loss of manganese, potassium and sodium is given in Table 6. Manganese in the forearm sweat of individual subjects ranged from 0.005-0.074 mcg/hr. The range of potassium and sodium from individual subjects was 9.78-62.51 mcg/hr and 9.95-57.9 mcg/hr, respectively. The lowest excretion of both sodium and potassium was for the same subject during the same treatment. Table 7 summarizes the available data on the cutaneous excretion of iron and zinc. Some of the data for these two minerals are missing because of contamination during processing. The range of excretion from the available data for the fifteen subjects was 0.014-0.529 mcg/hr for iron and 0.10-3.71 mcg/hr for zinc. There was no consistent trend of mineral excretion. No subject excreted consistently high or low levels of all minerals.

When examining the results summarized in Tables 5 - 7, it is apparent that the standard deviations are large. This is not unusual and other researchers have also reported

Table 6.

Average excretion^a of manganese, potassium, and sodium
in forearm sweat during three levels of intakes^b
and for three groups of subjects

Subjects	Intake of Mn ^b		Intake of K ^b		Intake of Na ^b				
	3.0	2.5	2.5	2.5	2182.7	3092.1	3294.2		
Group A	0.060 +0.01 —	0.032 +0.02 —	0.053 +0.01 —	32.052 +8.67 —	14.668 +4.36 —	35.281 +16.70 —	36.012 +6.81 —	16.132 +1.95 —	31.642 +10.90 —
Group B	0.032 +0.01 —	0.042 +0.02 —	0.021 +0.02 —	30.558 +6.59 —	31.506 +18.82 —	14.666 +4.25 —	29.052 +8.01 —	28.670 +15.36 —	13.386 +4.20 —
Group C	0.012 +0.01 —	0.032 +0.01 —	0.037 +0.01 —	25.806 +9.07 —	38.058 +7.58 —	31.756 +9.68 —	24.370 +15.92 —	40.734 +10.84 —	35.660 +10.27 —
Overall Mean	0.035 +0.02 —	0.035 +0.00 —	0.037 +0.20 —	29.472 +9.17 —	28.077 +12.07 —	27.235 +11.03 —	29.811 +5.86 —	28.512 +12.32 —	26.896 +11.96 —

^aMineral excretion is given in micrograms lost per hour + SD

^bIntake is given in milligrams per day

Table 7.

Average excretion^a of iron and zinc
in forearm sweat during three levels of intakes^b
and for three groups of subjects

Subjects	Intake of Fe ^b			Intake of Zn ^b		
	30.2	32.0	32.0	4.2	8.2	9.7
Group A	0.425 +0.12 —	— ^c	0.159 +0.20 —	1.650 +0.72 —	— ^c	1.102 +0.60 —
Group B	0.071 +0.08 —	0.207 +0.37 —	— ^c	0.504 +0.26 —	0.316 +0.22 —	— ^c
Group C	— ^c	0.094 +0.10 —	0.127 +0.07 —	— ^c	0.567 +0.10 —	1.263 +1.38 —
Overall Mean	0.248 +0.25 —	0.151 +0.08 —	0.143 +0.02 —	1.077 +0.81 —	0.440 +0.18 —	1.180 +0.11 —

^aMineral excretion is given in micrograms lost per hour + SD

^bIntake is given in milligrams per day

^cValues from this period unavailable due to contamination during processing

considerable variability between subjects (23, 8, 9). It is well documented that sweat rate differs between individuals and the area of the body on the same individual. Consolazio et al. (9) found that some of his adult male subjects did not sweat appreciably on the arms but their total body sweat volume was equivalent to other subjects. In the present study it was evident that some of the subjects sweated more than others. This was obvious by the varying amounts of moisture collected on the inside of the arm bag after it had been worn for one hour. This difference in sweat rate on the forearm could be one factor in the wide range of results found in this study.

The mineral excretion in forearm sweat of three groups of subjects on three dietary treatments were analyzed using an analysis of variance. With a 5% significance level it was found that the dietary treatment or intake of the mineral had no affect on its excretion in the sweat. The difference in the excretion of Ca, Cu, Mg, Mn, Na, and K for three intakes of each mineral was not statistically significant. The intakes of Cu and Mn between treatment A, B, and C did not differ greatly and a difference in excretion because of intake was not expected for these minerals. Because of the missing data, an analysis of variance was not attempted with iron and zinc. The data for these two minerals showed no consistency in showing higher

excretion during periods of higher intake. It would therefore seem probable that the dietary intake does not affect excretion of Fe and Zn in the sweat.

Excretion at Three Different Times

Because the mineral intake had no apparent effect on cutaneous excretion, the losses during each collection period were examined. The grouping of the subjects and the treatments were disregarded and the mean mineral excretion of all fifteen subjects for each collection was determined (Table 8). The lowest means for Cu, Mg, Mn, K and Na were reported during the second collection period. The data on zinc and iron from the second period are missing so it is not known whether or not they were lower for this period. The lower values during the second collection period could possibly be due to a change in activity. During collection periods 1 and 3 the subjects were allowed to participate in active play while wearing the arm bag. The subjects were allowed to play in the kindergarden facilities both indoors and out during the first and last collection periods. Because of bad weather, they were not able to participate in the same type activities during the second collection period, but were entertained quietly by watching television. This decrease in activity during the second period could have resulted in a reduced sweat rate and therefore lower mineral excretion during this period. A relationship

Table 8.

Mineral excretion in forearm sweat
at three different times

Period	Ca mcg/hr	Cu mcg/hr	Fe mcg/hr	Mg mcg/hr	Mn mcg/hr	K mcg/hr	Na mcg/hr	Zn mcg/hr
Period 1 (July 2)	16.821 +4.71 —	0.126 +0.02 —	0.253 +0.15 —	2.310 +0.75 —	0.046 +0.01 —	31.776 +0.27 —	33.441 +4.14 —	1.076 +0.69 —
Period 2 (Aug. 4)	9.424 +1.49 —	0.125 +0.01 —	— ^a	0.799 +0.05 —	0.023 +0.01 —	14.942 +0.47 —	17.964 +5.72 —	— ^a
Period 3 (Aug. 15)	6.482 +2.48 —	0.204 +0.10 —	0.108 +0.05 —	1.040 +0.57 —	0.039 +0.01 —	34.638 +3.79 —	33.816 +6.13 —	0.724 +0.33 —

^aData missing for this period

between increased activity and increased dermal mineral excretion has been found by other researchers (7, 28). It is well known that the amount of water and other nutrients lost through the skin depends on the rate of sweating which is influenced by activity and environmental temperature. Environmental temperature (Table 3) differed only by 3° F between the collection periods with the highest temperature of 71° F reported during the second collection period. Relative humidity was lowest during the second collection period which could also be a factor causing lower excretion during period 2. Sweat rates could have been higher during periods 1 and 3 when relative humidity was higher.

The means of Ca, Fe, Mg, Mn and Zn were higher during the first collection period. During this first period the subjects were unaware as to how sweat collections would be made. This nervous apprehension associated with a new and unfamiliar experience could have caused an increase in the sweat rate thus causing higher mineral excretions during this period. Nervous stimulation has been shown to increase sweat rate (5).

Comparison to the Literature

The results obtained using preadolescent girls in this study is compared to the results obtained by other researchers using adult subjects (Table 9). The means of three collections from each of fifteen subjects for Ca, Cu,

Table 9.

The mean and range of excretion of minerals
in forearm sweat as compared to literature values

Source of Data	Ca mg %	Cu mg %	Fe mg/L	Mg mg %	Mn mcg %	Na mEq/L	K mEq/L	Zn mg %
Mean of Present Study	1.09	0.015	0.186	0.140	3.6	1.22	0.685	0.087
Range of Present Study	0.34- 3.53	0.004- 0.06	0.014- 0.529	0.00- 0.798	0.5- 7.4	0.24- 2.69	0.25- 1.63	0.01- 0.37
Mitchell, ^b (1949), <u>20</u>	0.40- 4.50 ^a	0.004- 0.008 ^a	1.000- 2.000 ^a	0.004- 0.400 ^a	3.2- 7.4			
Seutter, ^b (1970), <u>24</u>	0.20- 52.1 ^a					34.0- 266.0 ^a	5.0- 59.0 ^a	
Johnston, ^b (1950), <u>17</u>	3.35		0.270					
Wheeler, ^b (1973), <u>28</u>			0.102- 0.35 ^a			2.62- 15.7 ^a	2.80- 7.17 ^a	
Dill, ^b (1933), <u>10</u>						1.00- 23.00 ^a		

^aRange of results

^bSee literature cited for reference

Mg, Mn Na, and K were found. The value for Fe and Zn are means of two collections from each of the fifteen subjects. The means and range of excretion found in this study are in the same magnitude as reported values. The range of Cu found in the present study was higher than the range reported in the literature. The only results on copper content of sweat were reported by Mitchell and Hamilton (20). Their experiments were done in the late 1940's and perhaps their method of copper detection was not as sensitive as present methods. This would account for the higher values found in the present experiment. The means of this study are within the range of results reported by Mitchell and Hamilton (20) for Ca, Mg, and Mn. The overall mean for iron excretion of the preadolescent girls (0.181 mg/L) is lower than the range of iron excretion found by Mitchell (20) but within the lower limits of the range found by Wheeler (28). In this study, sweat was collected from the forearm which would include only secretions from the eccrine glands. Other researchers (20, 28, 17) collected sweat from the entire body which possibly contained a higher proportion of perspiration secreted by the apocrine glands. It is known that iron is present in the secretions from the apocrine glands and if secretions from the apocrine glands were included in this study, the values would have possibly been higher. It is also possible

that less iron is excreted in the sweat of children than in the sweat of adults. Because children are in the growing state iron may be retained to meet growth requirements and less is excreted. Hussian (15) found that women suffering from hypochromic anaemia excreted less iron in sweat than normal women. Perhaps growing children who need iron for growth retain more iron by excreting less through the skin than do adults who are not growing.

Sodium excretion was lower in this study than in others. Other researchers (24, 28) collected sweat samples while their subjects were exercising heavily or exposed to high environmental temperatures. Wheeler (28) found that the Na excreted in the sweat increased as activity increased but the Na excreted in the urine decreased. The total excretion of Na (sweat + urine) did not increase as activity increased. In the present study more Na could have been excreted in the urine resulting in less excretion in the sweat.

The other researchers given in Table 9 (20, 24, 17, 28, 10) worked with adults and sweat was collected by increasing environmental temperature or activity until sweating was induced. It would seem from the results obtained from the preadolescent girls in this study, that the cutaneous mineral excretion of Ca, Cu, Mg, Mn, and Zn in preadolescent girls under normal atmospheric conditions are equivalent to

the amounts lost by adults. The dermal loss of Fe, Na and K in the sweat of these preadolescent girls was less than the amounts lost by adults.

SUMMARY AND CONCLUSIONS

An experiment was carried out to determine the cutaneous mineral excretions of preadolescent girls. The influence of mineral intake on excretion in the sweat was also studied.

Fifteen healthy preadolescent girls from the Blacksburg, Virginia region were the subjects for a metabolic study beginning July 12, 1973, and ending August 15, 1973. The subjects were randomly divided into three groups and were given three different dietary treatments. The mineral intake was determined by analyzing a composite of all the menus for each treatment.

Sweat collections for mineral analysis were taken in a polyethylene freezer bag which covered the hand and forearm of the subjects. The bag was worn by the subject for one hour and then the arm and bag were rinsed with a laural alcohol detergent solution and ion free water. The rinse solution was filtered and a sample was taken for analysis. The sample was evaporated to dryness and then wet ashed. Mineral determinations were made on a Perkin Elmer Model 305 (Three Slot Burner Head) Atomic Absorption Spectrophotometer.

There was not significant correlation between dietary intake of Ca, Cu, Mg, Mn, K, or Na and their excretion in the forearm sweat. There was considerable variation in excretion of Ca, Cu, Fe, Mg, Mn, Na, K and Zn in the forearm

sweat among subjects and within the same subject at different times.

The mean excretion from the fifteen preadolescent girls during three collection periods showed the following mineral excretion in the sweat: 1.09 mg % of Ca; 0.015 mg % of Cu; 0.186 mg/L of Fe; 0.140 mg % of Mg; 3.6 mcg % of Mn; 1.22 m Eq/L of Na; 0.685 m Eq/L of K; and 0.087 mg % of Zn. These results are in accordance with those found by other researchers using adult subjects.

It is suggested that additional studies with children be conducted to better understand the cutaneous mineral losses. More information is needed before the mineral retention of the growing child can be determined. Accurate methods of determining mineral loss from the entire body need to be found.

LITERATURE CITED

1. Adams, W. S., A. Leslie and M. H. Levin 1950 The dermal loss of iron. *Proc. Soc. Exper. Biol. & Med.* 74: 46.
2. Amatruda, T. T. and L. G. Welt 1953 Secretion of electrolytes in thermal sweat. *J. Appl. Physiol.* 5: 759.
3. Collins, K. J. and J. S. Weiner 1962 Observations on arm-bag suppression of sweating and its relationship to thermal gland fatigue. *J. Physiol.* 161: 538.
4. Collins, K. J., R. P. Eddy, A. Hibbs, A. L. Stock and E. F. Wheeler 1969 Nutritional and environmental studies on an ocean-going oil tanker. *Br. J. Indust. Med.* 28: 246.
5. Comar, C. L. and F. Bronner 1960 *Mineral Metabolism*. Vol. I, Academic Press, New York, p. 350.
6. Conn, J. W., L. H. Louis and B. F. Steele 1946 Hand sweat values in the calculation of chloride and nitrogen balance under conditions of hard work in humid heat. *Fed. Proc.* 5: 234.
7. Consolazio, C. F., L. O. Matoush, R. A. Nelson, L. R. Hackler and E. E. Preston 1962 Relationship between calcium in sweat, calcium balance, and calcium requirements. *J. Nutr.* 78: 78.
8. Consolazio, C. F., L. O. Matoush, R. A. Nelson, R. S. Harding and J. E. Canham 1963 Excretion of sodium, potassium, magnesium and iron in human sweat and the relation of each to balance and requirements. *J. Nutr.* 79: 407.
9. Consolazio, C. F., L. O. Matoush, R. A. Nelson, G. J. Isaac and J. E. Canham 1966 Comparisons of nitrogen, calcium and iodine excretion in arm and total body sweat. *Am. J. Clin. Nutr.* 18: 443.
10. Dill, D. B., B. F. Jones, H. T. Edwards and S. A. Oberg 1933 Salt economy in extreme dry heat. *J. Biol. Chem.* 100: 755.

11. Dill, D. B., F. G. Hall and H. T. Edwards 1938
Composition of sweat during acclimatization to
heat. *Am. J. Physiol.* 123: 412.
12. Freyberg, R. H. and R. L. Grant 1937 Loss of minerals
through the skin of normal humans when sweating is
avoided. *J. Clin. Invest.* 16: 729.
13. Food and Nutrition Board: Recommended Dietary
Allowances, Revised 1968. Washington, D. C.
National Academy of Sciences. Publication 1964.
National Research Council, Washington, D. C.
14. Foy, H. and A. Kondi 1957 Anaemias of the tropics:
Relation to iron intake, absorption and losses
during growth, pregnancy and lactation. *J. Trop.
Med. Hyg.* 60: 105.
15. Hussian, R. and V. N. Patwardhan 1959 Iron content of
thermal sweat in iron-deficiency anaemia. *Lancet*
276: 1073.
16. Issaksson, B., B. Lindholm and B. Sjogren 1967 A
critical evaluation of the calcium balance technic:
dermal calcium losses. *Metabolism* 16: 303.
17. Johnston, F. A., R. J. McMillan and E. R. Evans 1950
Perspiration as a factor influencing the require-
ments for calcium and iron. *J. Nutr.* 42: 285.
18. Ladell, W. S. S. 1948 The measurement of chloride
losses in the sweat. *J. Physiol.* 107: 465.
19. Mitchell, H. H. and M. Edman 1962 Nutritional
significance of the dermal losses of nutrients in
man, particularly of nitrogen and minerals. *Am.
J. Clin. Nutr.* 10: 163.
20. Mitchell, H. H. and T. S. Hamilton 1949 The dermal
excretion under controlled environmental conditions
of nitrogen and minerals in human subjects, with
particular reference to calcium and iron. *J. Biol.
Chem.* 178: 345.
21. Robinson, S. and A. H. Robinson 1954 Chemical
composition of sweat. *Physiol. Rev.* 34: 202.

22. Ryan, V. C. 1971 The cutaneous loss of calcium and magnesium in preadolescent girls. Masters Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
23. Schwartz, I. L. and J. H. Thaysen 1956 Excretion of sodium and potassium in human sweat. J. Clin. Invest. 35: 114.
24. Seutter, E., N. G. Groot, A. H. M. Sutorius and E. J. Urselmann 1970 The quantitative analysis of some constituents of crude sweat. Dermatologica. 141: 226.
25. Van Heyningen, R. and J. S. Weiner 1952 A comparison of armbag sweat and body sweat. J. Physiol. 116: 395.
26. Walker, A. R. and B. D. Richardson 1964 Growth rate in relation to calcium losses through sweat. Am. J. Clin. Nutr. 15: 309.
27. Weiner, J. S. 1945 The regional distribution of sweating. J. Physiol. 104: 32.
28. Wheeler, E. F., H. El-Neil, J. O. Willson, and J. S. Weiner 1973 The effect of work level and dietary intake on water balance and the excretion of sodium, potassium and iron in a hot climate. Br. J. Nutr. 30: 127.

Appendix A

A Brief Description of

Whole Body Sweat Collection and Analysis

Whole Body Sweat Collections and Nitrogen Determinations

Sweat collections from the whole body were made during the last two days of the experimental period by the following procedure:

First Day of Sweat Collections

Each subject was bathed (including hair) in 4 L of a 0.005% laural alcohol detergent solution and 3 L of tap water. This water was discarded. Following the bathing, the subjects were dressed in nitrogen free absorbant clothing.

Second Day

The absorbant clothing was removed and each subject was bathed a second time with 4 L of detergent solution and 3 L of water. This water was saved and to it was added the clothes worn the preceding day. Following the second bath, the subjects were dressed in a second set of nitrogen free clothing.

Third Day (First Day of Next Period)

The second set of clothing was removed and the subjects were bathed a third time by the same procedure as baths one and two. The second set of clothing was added to this bath water. The bed sheets used by the subjects during the collection were also collected and were soaked by the same method as the clothing.

The clothing and sheets were soaked in an acidified solution until equilibrium had been reached. A 500 ml aliquot was then removed, filtered and evaporated to approximately 50 ml. Nitrogen determinations were made on this concentrated 50 ml sample using the Kjeldahl-Gunning-Arnold method with selenium and copper catalysts.

VITA

Coleen Hogston Walls, daughter of Mr. and Mrs. Charles W. Hogston, was born in Saltville, Virginia on July 1, 1950. From 1968 to 1972 she attended Virginia Polytechnic Institute and State University where she earned a Bachelor of Science Degree in Human Nutrition and Foods.

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Coleen Hogston Walls

CUTANEOUS MINERAL LOSS
IN PREADOLESCENT GIRLS

by

Coleen Hogston Walls
(ABSTRACT)

The excretion of Ca, Cu, Fe, Mg, Mn, Na, K and Zn in the sweat of 7-10 year old girls living in southwest Virginia was investigated. Sweat samples were collected in an arm bag which covered the hand and forearm of the subject. The samples were wet ashed and assayed on an Atomic Absorption Spectrophotometer.

No significant correlation was found between mineral intake and excretion in the forearm sweat. There was no apparent relationship between the environmental temperature and the excretion of minerals in the sweat. There were great variations in mineral excretion among subjects. Calcium excretion ranged from 3.39-35.32 mcg/hr. Copper and magnesium excretion in the forearm sweat ranged from 0.04-0.66 mcg/hr and 0.00-7.98 mcg/hr, respectively. The range of excretion for the other minerals studied was: 0.005-0.074 mcg/hr of Mn; 9.78-62.51 mcg/hr of K; 9.95-57.9 mcg/hr of Na; 0.014-0.529 mcg/hr of Fe; and 0.10-3.71 mcg/hr of Zn. Reasons for discrepancies between these results and those reported in the literature are discussed. Suggestions for further research on the cutaneous mineral excretion of the preadolescent age group are recommended.